Cell Sheet Engineering for reproducing the Bone Marrow Hematopoietic Stem Cell Niche

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Hematopoietic stem and progenitor cells (HSPC) are multipotent cells responsible for the maintenance and renewal of the hematopoietic lineage in the adult body. The fate of these stem cells is closely regulated by their surrounding microenvironment, or niche. The importance of the microenvironment for HSPC function has been long recognized by researchers that more than 30 years ago attempted to emulate it in 2D using a layer of bone marrow stromal cells to culture hematopoietic cells for long time periods (Dexter-type cultures). However, all the models based on feeder layers are less than perfect in recreating the hematopoietic microenvironment. The use of growth factor cocktails provided some promising results concerning the maintenance and proliferation of some cell populations but still struggle to deliver the correct microenvironment for the maintenance of suitable HSPC populations. Part of the problem of the current systems lies on the lack of the third dimension. At the same time, the proposed three-dimensional methodologies using scaffolds to engineer the bone marrow (BM) microenvironment present very limited results probably due to the scaffolding matrices' intrinsic limitations. Therefore, an engineered BM microenvironment capable of acting as a functional HSPC niche would provide a tremendous tool for the study of hematopoiesis as well as for obtaining and maintaining HSPC. Using osteogenic cell sheets, we have previously demonstrated that it was possible to induce the ectopic formation of mature bone tissue with a clear bone marrow, avoiding the use of scaffolds.

In the present work, we studied the potential of using osteogenic cell sheets to build *in vitro*, a 3D microenvironment capable of providing HSPC a suitable niche for their survival and proliferation. For this, we used bone marrow stromal cells and adipose-derived stem cells to produce the osteogenic cell sheets and human umbilical cord blood as a source of hematopoietic stem cells.